Field Screening of Experimental Corn Hybrids and Inbred Lines for Multiple Ear-Feeding Insect Resistance

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ABSTRACT Identifying and using native insect resistance genes is the core of integrated pest management. In this study, 10 experimental corn, *Zea mays* L., hybrids and 10 inbred lines were screened for resistance to major ear-feeding insects in the southeastern Coastal Plain region of the United States during 2004 and 2005. Ear-feeding insect damage was assessed at harvest by visual damage rating for the corn earworm, *Helicoverpa zea* (Boddie), and by the percentage of kernels damaged by the maize weevil, *Sitophilus zeamais* Motschulsky, and stink bugs [combination of *Euschistus servus* (Say) and southern green stink bug, *Nezara viridula* (L.)]. Among the eight inbred lines and two control populations examined, C3S1B73-5b was resistant to corn earworm, maize weevil, and stink bugs. In contrast, C3S1B73-4 was resistant to corn earworm and stink bugs, but not to maize weevil. In a similar manner, the corn hybrid S1W*CML343 was resistant to all three ear-feeding insects, whereas hybrid C3S1B73-3*Tx205 was resistant to corn earworm and maize weevil in both growing seasons, but susceptible to stink bugs in 2005. The silk-feeding bioassay showed that corn earworm developed better on corn silk than did fall armyworm. Among all phenotypic traits examined (i.e., corn ear size, husk extension, and husk tightness), only corn ear size was negatively correlated to corn earworm damage in the inbred lines examined, whereas only husk extension (i.e., coverage) was negatively correlated to both corn earworm and maize weevil damage on the experimental hybrids examined. Such information could be used to establish a baseline for developing agronomically elite corn germplasm that confers multiple ear-feeding insect resistance.

KEY WORDS *Helicoverpa zea*, *Sitophilus zeamais*, stink bugs, husk extension, silk-feeding bioassay

The key ear-feeding insects limiting corn, *Zea mays* L., yield in Georgia and other southern states of the United States are earworm, *Helicoverpa zea* (Boddie), and fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae); maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae); and stink bugs (Heteroptera: Pentatomidae). Several corn germplasms conferring resistance to individual ear-feeding insects have been developed in recent decades. However, few reports examined the possibility of multiple ear-feeding insect resistance in corn germplasm.

When plants are at the silking stage, the corn earworm oviposits its eggs on corn silks. Small larvae feed on the silks and larger larvae subsequently feed on young developing kernels. Fall armyworm also may oviposit on corn silks and feed on silks and developing kernels. Maysin, a flavone glycoside, was originally identified as an important allelochemical in corn silk that conferred resistance to insect feeding, but other compounds with antibiotic activity in corn silk, such as apimaysin, chlorogenic acid, and iso-orientin, also have been identified (Waiss et al. 1979, Gueldner et al. 1991, Wiseman et al. 1992, Widstrom and Snook 1998, Lynch et al. 2003). Larval weight of corn earworms was negatively correlated to the concentration of maysin in corn silk in laboratory bioassays, although larvae might avoid feeding on corn silk with a high level of maysin if the husk was not tight in the field (Wiseman and Isenhour 1992, Wiseman and Widstrom 1992). Avoidance of kernel feeding by the corn earworms also has been observed, whereby some varieties have a long, fairly tight silk channel with a large amount of silk that can limit corn earworm movement and feeding on the silk channel area and developing kernels (Wiseman et al. 1972, 1978). Physical traits of the ear husk (e.g., long and tight corn husk) also influence

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corn earworm damage by acting as a barrier to prevent or delay larval feeding on developing kernels (Wiseman et al. 1977, Archer et al. 1994, Rector et al. 2002).

Stink bugs are a group of piercing–sucking insects that feed on the developing ears and kernels by penetrating through corn husks. Several species attack corn, but the main species are the brown stink bug, *Euschistus servus* (Say), and the southern green stink bug, *Nezara viridula* (L.). Both species attack corn during ear formation. After pollination, they also feed through the husk on individual kernels. Kernel feeding reduces kernel weight and quality, and it may provide a point of entry for fungal pathogens.

The maize weevil is one of the most serious postharvest insect pests of maize storage in the tropical and subtropical areas of the world. In the southeastern United States, the weevil infestations naturally occur in maturing maize plants before harvest (Brown and Lee 2002, Kim and Kossou 2003), and resistant hybrids have been recommended as one of the most promising methods of minimizing damage caused by the maize weevil in grain storage facilities (Kim and Kossou 2003). But, preharvest resistance to maize weevil is still not well understood in corn. The standard screening method is to artificially infest dried grain under controlled storage conditions with constant temperature and relative humidity (Widstrom et al. 1974, Kim and Kossou 2003). This method may not effectively screen for maize weevil resistance in perharvest cornfields, because weevils migrate to maturing corn plants when the kernel moisture levels are at 30% (X.N., unpublished data). In addition, ear- or fruit-feeding insect damage also facilitates the entry of mycotoxin-producing fungi, and in turn, contributes to high levels of mycotoxins in corn and other crops (Widstrom et al. 1975; Dowd 1998, 2003). Thus, multiple ear-feeding insect resistance in corn may be crucial in improving both yield and quality of corn production by simultaneously reducing both insect damage and mycotoxin contaminations.

To evaluate corn resistance to multiple ear-feeding insects, 10 experimental hybrids and 10 inbred lines were examined for 2 yr (2004 and 2005) under field conditions in Tifton, GA. The ear-feeding insects included both chewing (i.e., the corn earworm and maize weevil) and piercing–sucking insects (i.e., the brown and the southern green stink bugs). To further examine the contribution of physical traits to ear-feeding insect resistance, husk coverage and ear size were measured, and correlations with insect damage also were examined. A silk-feeding bioassay also was conducted in the laboratory to assess corn silk resistance to corn earworm and fall armyworm neonates, because larvae of both species commonly infest developing corn ears (including both field corn and sweet corn) in the southeastern Coastal Plain region of the United States.

Materials and Methods

Plants and Insects. Ten inbred lines or populations and 10 experimental hybrids were used in the experiments in 2004 and 2005. Inbred lines Tx204, C3S1B73-3, C3S1B73-4, C3S1B73-5b, C3S1B73-7, C3S1B73-8, B76C9C, and S1W were developed by the corn breeding program at Texas A&M University, Lubbock, TX, and they have been selected for desirable agronomic traits and above-average earworm resistance in the breeding nursery. Populations EPM6 and SIM6 (Widstrom and Snook 2001c) were used as positive (resistant) controls, because they contain high levels of maysin in corn silk that confer corn earworm resistance. Eight experimental hybrids we used in this study were from the corn breeding program at Texas A&M University. They were S1W*CML343, C3W64A-1*B110, Tx205*B110, C2A554-1*B110, C3A654-1*B110, C3S1B73-3*Tx204, C3S1B73-3*Tx205, SGP3*C3S1B73-3. Two commercial hybrids, Pioneer hybrid 31B13 (P3223+Bt) and 3223 (non-*Bacillus thuringiensis* [Bt]), served as resistant and susceptible controls for the study, respectively. Corn earworm and fall armyworm eggs and neonates used in this study were from the colonies maintained at the Insectary of the Plant Protection and Management Research Unit, USDA–ARS, Tifton, GA.

All experiments were conducted under the field conditions in 2004 and 2005 growing seasons at the Belflower Agricultural Research Farm, USDA–ARS, Tifton, GA. Field experiments used a randomized complete block design with four replications of the plant entries. The experimental plot was 3 by 1 m, and the first ears from five or 10 plants were sampled from each plot for all insect damage ratings.

Insect Infestation and Damage Ratings. Although corn earworm damage occurs consistently at Tifton, GA, location, we still artificially infested the experimental plots with corn earworm eggs to establish a uniform infestation to screen for insect resistance. Corn earworm damage rating was based on manual infestation of fresh corn silk by using two to five near-hatch corn earworm eggs. All corn ears in a plot were infested at full silk stage within a 7-d period. Both cob and developing kernel damage by corn earworm feeding was rated according to the scale by Widstrom (1967), where 0 is no damage, 1 is only silk damage and 0 cm of cob damage; and 2 and up = 1+ centimeter(s) of insect feeding penetration (or tunneling) on a developing corn cob, which included damage on the developing cob and young kernels. Both fall armyworm and corn earworm could naturally infest corn ears in a field. Given that we artificially infested all plants with corn earworm eggs when the plants were at full silk stage, ear damage caused by the fall armyworm would have been minimal, because of the cannibalistic nature of corn earworm larvae, which led to normally only one larva surviving on a corn ear. Therefore, we consider the chewing insect penetration damage on developing corn ears was mainly caused by the corn earworms.

Kernel damage by maize weevil and stink bugs was assessed based on natural infestations. Maize weevil damage was recorded at harvest time with the percentage of kernels with weevil emergence holes. Stink bug damage on the sampled corn ears also was recorded as the percentage of discolored kernels from each sampled ear. Damage was assessed using five to 10 ears randomly selected from each experimental plot. Although natural infestations of corn earworm, maize weevil, and stink bugs occurred every year, the severity of infestations and damage of different specie of ear/kernel-feeding insect did vary from year to year. Corn earworm and maize weevil damage was evaluated in both 2004 and 2005, while stink bug damage was only assessed in 2005, because low natural infestation of stink bugs in our experimental plots prevented meaningful assessments in 2004.

Husk Coverage and Ear Size Examination. Husk coverage was evaluated in this study using husk extension to understand the correlation of ear coverage and insect damage. Husk extension was the distance (centimeters) between the top of a corn ear and the tip of the husk, which was different from the husk tightness. Husk tightness was the index to assess the physical tightness of the husk wrapping around a corn ear (Wiseman and Isenhour 1992, Rector et al. 2002). Because previous studies have evaluated the effect of husk tightness on insect damage, the current study only evaluated husk extension (or coverage) in relation to ear-feeding insect damage. Furthermore, the size of the first ear on a plant was assessed using the number of kernels per ear to estimate the yield potential of a given corn inbred line or hybrid. Kernel number per ear was estimated by counting the number of kernels in a row and a column of a sampled corn ear. Although the husk extension was measured on all of the sampled (5–10) corn plants, the kernel number was estimated only on a randomly selected representative ear per experimental plot. All data were collected from the first ears of all sampled plants.

Corn Silk-Feeding Bioassays. Fresh corn silks were collected from each of the 10 inbred lines and populations in the field experiments. Field-collected silk (5 g) was placed in insect rearing cups lined with a piece of moistened paper towel. The corn silk from eight inbred lines, Tx204, C3S1B73-3, C3S1B73-4, C3S1B73-5b, C3S1B73-7, C3S1B73-8, B76C9C, S1W, were used in the bioassay. In addition, the silk from EPM6/SIM6 (i.e., the combination of silk from EPM6 and SIM6 with high level of maysin) and Hytest7930Bt was used as the controls. Because both corn earworm and fall armyworm larvae could feed on various tissues of a corn ear (i.e., silks, cobs, and developing kernels), the neonates of both corn earworm and fall armyworm were used in the corn silk-feeding bioassay. A neonate was placed in a cup with fresh silk from a corn inbred line. The experiment was maintained in a rearing room at 25°C, 70% RH, and a photoperiod of 14:10 (L:D) h. The corn silk was changed 7 d after the initiation of the experiment, and larval survival and weight were recorded 15 d after the initiation of the experiment. The laboratory corn silk-feeding bioassays were conducted as a randomized complete block design with 10 replications for each of the 10 corn inbred lines and two insect species were considered the block factor.

Assessment Criteria and Data Analysis. The criteria for designation of multiple insect resistance in the experimental entries included three components: 1) the designated entries had the least insect feeding damage according to statistical analysis; 2) the damage rating of the designated entries should be at or lower than the mean damage ratings from all entries; and 3) the entries consistently demonstrated low insect damage ratings in both 2004 and 2005 growing seasons.

All ear-feeding insect (i.e., corn earworm, maize weevil, and stink bugs) damage and plant phenotypic trait (i.e., husk extension and kernel numbers per ear) data were recorded in the field before harvest. All data collected were subjected to the analysis of variance (ANOVA) by using PROC GLM followed by least significant difference (LSD) ($\alpha = 0.05$) for mean separation (SAS Institute 2003). Percentage data of maize weevil and stink bug damage were transformed before analysis using square-root transformation. The correlation between damage by the three insects, husk extension, and kernel numbers also were examined using Pearson's correlation coefficient using PROC CORR procedure of the SAS software (SAS Institute 2003). All data used in the correlation procedure were the combined results from the 2-yr study.

Results

Corn Earworm and Maize Weevil Damage on Corn Inbreds. Corn earworm damage ratings were significantly different among the inbred lines ($F = 6.56$; df = 9, 559; $P = 0.0001$) but not between years ($F = 0.05$; df = 1, 613; $P = 0.8281$). Corn earworm damage also was significantly affected by entry and year interaction ($F = 4.4$; df = 9, 559; $P = 0.0001$); thus, data were presented by year (Fig. 1A and B). Inbred line C3S1B73-4 and C3S1B73-5b consistently showed less corn earworm damage than the rest of the entries. The resistance level was close to the control population, SIM6 (Fig. 1A and B).

The percentage of maize weevil-damaged kernels was not significantly different ($P > 0.05$) among the inbred lines, years, or the two-way interaction (data not shown). Nevertheless, EPM6, C3S1B73-7, C3S1B73-5b had the least (0.7, 1, and 1.2%) amount of weevil damage, whereas C3S1B73-3 had the most weevil damage (7%).

Corn Earworm and Maize Weevil Damage on Experimental Corn Hybrids. Corn earworm damage ratings were significantly different among the 10 experimental hybrid entries ($F = 6.69$; df = 9, 713; $P = 0.0001$) between the 2 yr ($F = 6.29$; df = 1, 713; $P = 0.0123$), and year by entry interaction ($F = 4.13$; df = 9, 713; $P = 0.0001$). Because of the significant interaction the data were presented by years, respectively (Fig. 2A and B). In both years, hybrid S1W*CML343 showed the least amount of corn earworm damage, which was statistically equivalent to the amount of damage on the Bt transgenic control, Pioneer hybrid 31B13 (Fig. 2A and B).

The percentage of maize weevil-damaged kernels in corn hybrids was not as high as in inbred lines. The percentage of weevil-damaged kernels was not significantly ($F = 2.49$; df = 9, 26; $P = 0.0681$) different among experimental hybrid entries, but it was signif-

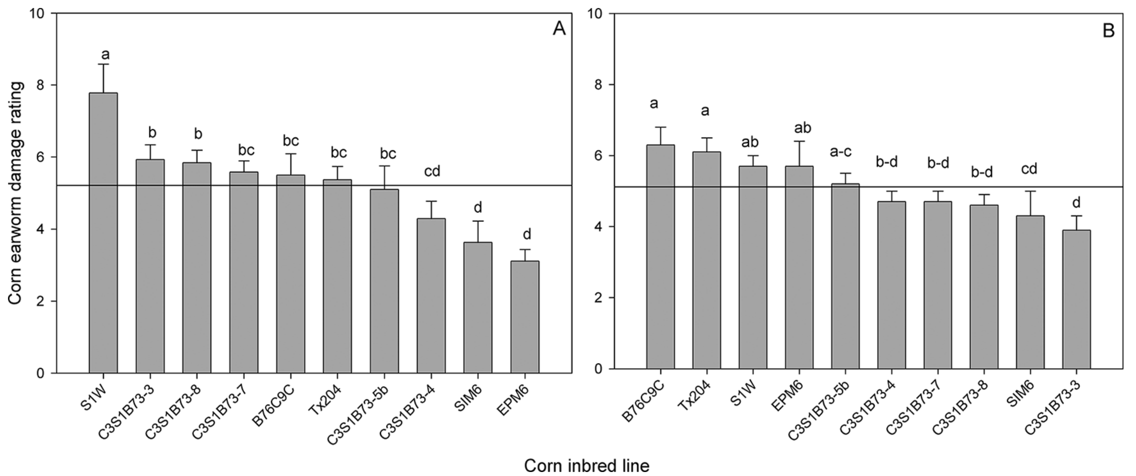

Fig. 1. Difference in corn earworm damage ratings on the 10 corn inbred lines. (A) Data from 2004. (B) Data from 2005. The bars with the same letters were not significantly different ($\alpha = 0.05$; LSD). The horizontal line on the graph indicates the mean damage rating of all entries.

icantly different between the 2 yr ($F = 14.18$; df = 1, 65; $P = 0.0004$). In 2004, Tx205*B110 and C3S1B73-3*Tx204 had >2% kernel damage, whereas entries S1W*CML343, C2A554-1*B110, C3A654-1*B110, and C3S1B73*Tx205 had <0.6% kernel damage. In 2005, S1W*CML343, C3A654-1*B110, C3S1B73-3*Tx204, C2A554-1*B110, and C3S1B73-3*Tx205 had the least damage (<0.3%), whereas C3W64A-1*B110 and SGP3*C3S1B73-3 had the most damage (>0.5%). The combined analysis showed that S1W*CML343, C2A554–1*B110, and C3A654-1*B110 had the lowest percentage of kernel damage (<0.3%), whereas Tx205*B110 and C3S1B73-3*Tx204 had the greatest percentage (>1%) of weevil damage (data not shown).

Assessment of Stink Bug Damage. High infestation levels of the brown and southern green stink bugs occurred on corn plants in 2005. The percentage of stink bug-discolored kernels was significantly different among inbred lines ($F = 2.06$; df = 9, 312; $P = 0.0300$) with inbred line C3S1B73-5b and SIM6 having the least amount of discolored kernels than the other entries (Table 1). When a similar analysis was performed on the data collected from the corn hybrids, S1W*CML343 and C2A554-1*B110 had significantly less ($F = 2.79$; df = 9, 369; $P = 0.0036$) damage than the rest of hybrid entries in this study (Table 1).

Corn Silk-Feeding Bioassays. Effect of corn silk on both fall armyworm and corn earworm development was not the same. Corn silk was a better food source

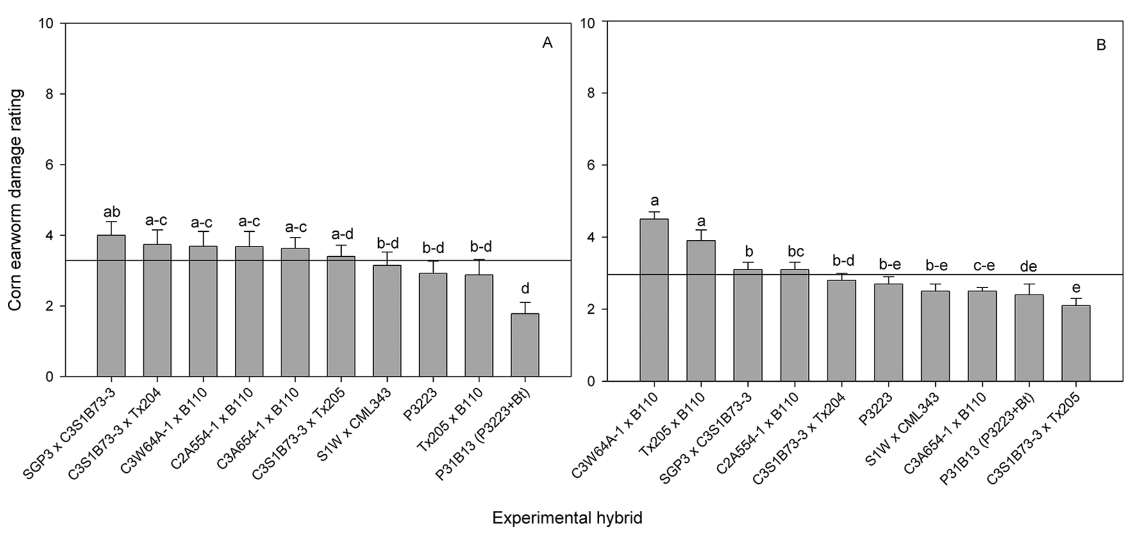

Fig. 2. Difference in corn earworm damage ratings on the experimental hybrids of corn. (A) Data from 2004. (B) Data from 2005. The bars with the same letters were not significantly different ($\alpha = 0.05$; LSD). The horizontal line on the graphs indicates the mean damage rating of all entries.

Table 1. Percentage of stink bug-damaged kernels on corn inbred lines and hybrids in 2005

Inbred entry	n^a	Damage (%)	Hybrid entry	n^a	Damage (%)
B76C9C	26	8.8 ± 2.3a	Tx205*B110	40	1.9 ± 0.4a
Tx204	39	6.4 ± 1.4ab	C3S1B73-3*Tx205	40	1.9 ± 0.4a
C3S1B73-8	23	5.5 ± 1.9abc	C3W64A-1*B110	41	1.7 ± 0.3ab
S1W	42	3.4 ± 0.7bc	SGP3*C3S1B73-3	40	1.7 ± 0.6abc
C3S1B73-3	29	2.5 ± 0.7cde	C3S1B73-3*Tx204	40	1.5 ± 0.4abc
C3S1B73-4	37	2.7 ± 0.5bcd	C3A654-1*B110	40	1.2 ± 0.4abc
EPM6	23	2.1 ± 0.4bcde	P31B13 (P3223+Bt)	39	1.0 ± 0.2bc
C3S1B73-7	37	2.1 ± 0.7cde	P3223	39	0.8 ± 0.2c
C3S1B73-5b	44	1.1 ± 0.3de	S1W*CML343	40	0.3 ± 0.2d
SIM6	27	1.0 ± 0.4e	C2A554-1*B110	40	0.2 ± 0.06d

Means with different letters within a column were significantly different after root-square transformation ($\alpha = 0.05$; LSD).
[a] n denotes the number of samples.

for corn earworm than for fall armyworm ($F = 77.02$; df = 1, 181; $P = 0.0001$). Corn earworms reared on corn silk were significantly larger (203.10 ± 12.23 mg) than fall armyworms (107.01 ± 6.30 mg), irrespective of the variation among the corn inbred lines. Silk from different inbred lines and controls also significantly ($F = 8.79$; df = 9, 181; $P = 0.0001$) affected the larval weight. The effect of corn silks on larval development was significantly ($F = 5.79$; df = 9, 181; $P = 0.0001$) differed between the two insect species. Only one of the 10 corn earworm larvae survived for 15 d on the silk of Hytest7930Bt. Weights of corn earworms after the 15-d feeding on corn silk from C3S1B73-4, C3S1B73-5b, C3S1B73-8, and B76C9C were not significantly different from the weight of larvae that had fed on the silks of the resistant control, EPM6/SIM6 (Fig. 3A). Also, the 15-d larval weight of corn earworms that had fed on the corn silk of C3S1B73-7 was the greatest among the 10 entries examined (Fig. 3A). In contrast, the 15-d larval weight of fall armyworms that had fed on the silk of B76C9C and EMP6/SIM6 was about half the weight of fall armyworms that had fed on silks of the other eight maize entries (Fig. 3B). Hybrid Hytest7930*Bt* had no effect on larval development of the fall armyworms (Fig. 3B), although Hytest7930*Bt* was effective in suppressing larval development of the corn earworms (Fig. 3A).

Ear Characteristics and Their Correlation with Insect Damage. Husk extension was significantly different among the corn inbred lines ($F = 5.32$; df = 9, 402; $P = 0.0001$) and between years ($F = 105.79$; df = 1, 402; $P = 0.0001$). Entry by year interaction also significantly influenced husk extension ($F = 6.83$; df = 9, 402; $P = 0.0001$). When husk extension was compared among experimental hybrids, it differed significantly among the hybrids ($F = 56.70$; df = 9, 713; $P = 0.0001$) and between the years ($F = 629.49$; df = 1, 713; $P = 0.0001$). The entry by year interaction was not significant ($P > 0.05$).

The number of kernels was used to estimate size of the ear, and to estimate the yield potential of each entry. The number of the kernels was significantly different among the corn inbred line entries ($F = 3.42$; df = 9, 25; $P = 0.0072$) and between years ($F = 12.63$;

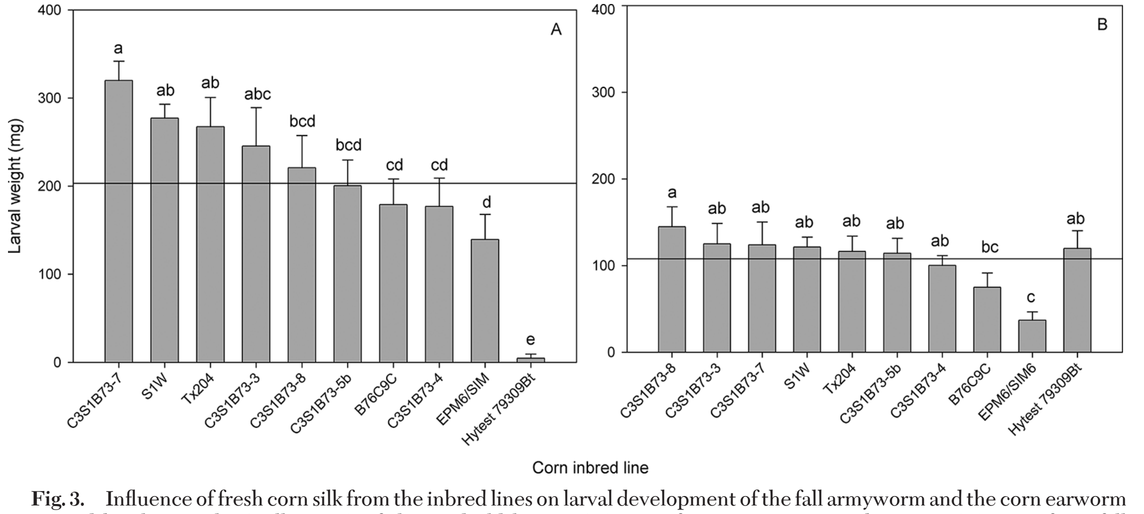

Fig. 3. Influence of fresh corn silk from the inbred lines on larval development of the fall armyworm and the corn earworm measured by the weight (milligrams) of the 15-d old larvae. (A) Data from corn earworm bioassay. (B) Data from fall armyworm bioassay. The bars with the same letters were not significantly different ($\alpha = 0.05$; LSD). The horizontal line on the graphs indicates the average larval weight among all inbred lines.

Table 2. Number of kernels per ear from corn inbred lines and hybrids used in 2004 and 2005 growing seasons[a]

Inbred line	2004	2005	Hybrid	2004	2005
C3S1B73-8	471 ± 48a	397 ± 16a	SGP3*C3S1B73-3	895 ± 79a	575 ± 28d
EPM6	471 ± 42a	399 ± 56a	Tx205*B110	865 ± 14ab	882 ± 38a
C3S1B73-4	451 ± 12ab	356 ± 17abc	C3S1B73-3*Tx205	756 ± 28bc	741 ± 42b
C3S1B73-3	440 ± 50ab	373 ± 28ab	C3S1B73-3*Tx204	755 ± 32bc	709 ± 12bc
C3S1B73-7	436 ± 37ab	430 ± 52a	C3A654-1*B110	733 ± 26c	691 ± 40bc
C3S1B73-5b	424 ± 42abc	376 ± 10ab	C3W64A-1*B110	720 ± 52c	718 ± 65bc
Tx204	383 ± 37abc	364 ± 20abc	P31B13 (P3223+Bt)	716 ± 34c	665 ± 52bcd
SIM6	367 ± 37abc	271 ± 31c	C2A554-1*B110	687 ± 31c	609 ± 24cd
B76C9C	349 ± 50bc	333 ± 22abc	P3223	680 ± 39cd	656 ± 24bcd
S1W	311 ± 30c	285 ± 48bc	S1W*CML343	567 ± 36d	695 ± 40bc

Means with different letters within a column were significantly different ($\alpha = 0.05$; LSD).
[a] Sample size $n = 23$–44 for inbred lines because of poor germination rate of seeds, whereas sample size $n = 39$–41 for the hybrids.

df = 1, 25; $P = 0.0015$), but the entry by year interaction was not significant ($P > 0.05$). Inbred line C3S1B73-8 had the greatest number of kernels among all inbred lines in both growing seasons (Table 2). Kernel number also differed significantly among the 10 hybrid entries ($F = 4.61$; df = 9, 26; $P = 0.0019$) and years ($F = 4.23$; df = 1, 26; $P = 0.0498$). The hybrid entry by year interaction also significantly influenced the number of kernels per ear ($F = 3.55$; df = 9, 26; $P = 0.0054$). The hybrid Tx205*B110 and C3S1B73-3*Tx204 had the greatest number of kernels per ear among the experimental hybrids examined in both years (Table 2). In contrast, hybrid 3GP3*C3S1B73-3 had the greatest number of kernels in 2004, but the least number of kernels in 2005. One of the possible causes for this observation would be the variation in environmental conditions between 2004 and 2005 at Tifton, GA. Although the 2004 growing season had the normal amount of precipitation, the 2005 season was abnormal. At the beginning of the 2005 growing season, the corn plants received an above-average amount of precipitation; however, the crop suffered hot and dry environmental conditions from flowering to harvest time.

Correlations between insect damage and phenotypic traits of inbreds and hybrids also were observed (Tables 3 and 4). Kernel number per ear was negatively correlated to corn earworm damage within the inbreds, but it was not correlated within hybrids. Kernel number was not correlated with maize weevil damage irrespective of the types of inbreds or hybrids used in the experiment. Corn husk extension of inbreds was not correlated with insect damage, but husk extension of hybrids was negatively correlated to corn earworm damage ($r = -0.2378$, $P = 0.0348$; $n = 79$) as well as to maize weevil damage ($r = -23946$, $P = 0.0335$; $n = 79$). Stink bug damage was not correlated with either husk extension or kernel number in inbred lines or experimental hybrids (Tables 3 and 4). All three types of ear-feeding insect damage (i.e., corn earworm damage ratings and the percentages of stink bug- and maize weevil-damaged kernels) were not correlated within either among the inbred lines or the hybrids examined (Tables 3 and 4).

Additional correlation analyses of hybrid entries showed a negative correlation ($r = -0.34$, $P = 0.0019$; $n = 80$) between the husk extension and corn earworm damage in C3A654-1*B110, C3S1B73-3*Tx205, and SGP3*C3S1B73-3 (Fig. 4A–C) but not on the other experimental hybrids. The hybrid C3S1B73-3*Tx205 (shown in Fig. 4B) had the least amount of damage among all three entries presented in Fig. 4A–C. The data from C3A654-1*B110 and SGP3*C3S1B73-3 showed that no insect damage occurred on ears with no husk extension (or coverage) (Fig. 4A and C), which indicated that other plant traits (e.g., high level of allelochemicals) might have contributed to their resistance to ear damage. In contrast, the data collected from C3S1B73-3*Tx205 indicated a

Table 3. Correlation between insect damage and phenotypic traits of the 10 inbred lines

Indices assessed	Kernel no.	CEW damage	MW damage	SB damage
Husk extension	$r = -0.16065$ $P = 0.1628$ $n = 77$	$r = 0.00357$ $P = 0.9754$ $n = 77$	$r = 0.18667$ $P = 0.1041$ $n = 77$	$r = 0.19870$ $P = 0.2317$ $n = 38$
Kernel no.		$r = -0.28968$[a] $P = 0.0106$ $n = 77$	$r = -0.15308$ $P = 0.1809$ $n = 78$	$r = -0.09636$ $P = 0.5649$ $n = 38$
CEW damage			$r = 0.19442$ $P = 0.0902$ $n = 77$	$r = 0.09443$ $P = 0.5728$ $n = 38$
MW damage				$r = -0.16486$ $P = 0.3226$ $n = 38$

CEW, corn earworm; MW, maize weevil; and SB, stink bugs.
[a] Coefficient value was statistically significant ($\alpha = 0.05$).

Table 4. Correlation between insect damage and phenotypic traits of the 10 experimental hybrids

Indices assessed	Kernel no.	CEW damage	MW damage	SB damage
Husk extension	$r = -0.17238$ $P = 0.1287$ $n = 79$	$r = -0.23781$[a] $P = 0.0348$ $n = 79$	$r = -0.23946$[a] $P = 0.0335$ $n = 79$	$r = -0.05978$ $P = 0.7177$ $n = 39$
Kernel no.		$r = 0.17841$ $P = 0.1157$ $n = 79$	$r = 0.19622$ $P = 0.0831$ $n = 79$	$r = -0.01879$ $P = 0.9096$ $n = 39$
CEW damage			$r = 0.08225$ $P = 0.4711$ $n = 79$	$r = 0.16198$ $P = 0.3245$ $n = 39$
MW damage				$r = 0.15509$ $P = 0.3458$ $n = 39$

CEW, corn earworm; MW, maize weevil; and SB, stink bugs.
[a] Coefficient value was statistically significant ($\alpha = 0.05$).

close correlation between husk coverage and the amount of corn earworm damage (Fig. 4B).

Discussion

Multiple insect resistance has been previously examined in recent years by a number of researchers on soybean [*Glycine max* (L.) Merr.] (All et al. 1989, Kraemer 2001), sorghum [*Sorghum bicolor* (L.) Moench] (Nwanze et al. 1991), corn (Abel et al. 2000), transgenic tomato (*Lycopersicon esculentum* Mill.) (Abdeen et al. 2005), sweetpotato [*Ipomoea batatas* (L.) Lam.] (Jackson and Bohac 2006), and turfgrasses (Anderson et al. 2006). All studies indicated that identification of multiple insect resistance on crop plants could be challenging, because the variation in plant tissues (or organs) on which different insect pests prefer to feed. In particular, Abel et al. (2000), using four corn insects feeding on leaf, ear, and stalk and Kraemer (2001) using two guilds of soybean insects feeding on leaf and seed concluded that insect resistance of a crop plant in vegetative and reproductive growth is likely to be regulated by different defense mechanisms.

In current study, we only focused on major ear-feeding insect complex. Among the eight inbred lines and two insect-resistant maize populations examined in this study, C3S1B73-5b showed less damage by all three insects (i.e., corn earworm, maize weevil, and stink bugs), and C3S1B73-4 showed less damage by corn earworm and stink bugs but not by maize weevil. C3S1B73-3, C3S1B73-4, C3S1B73-5b, C3S1B73-7, and C3S1B73-8 are sister lines derived from a breeding population from a cross between B73 and S1W*CML343. During the process of developing these inbred lines, the selection effort was focused on desirable agronomic traits and resistance to earworm (W.X., unpublished data). Hybrid S1W*CML343 showed less damage by all three ear-feeding insects, whereas the hybrid C3S1B73-3*Tx205 showed resistance to the corn earworm and maize weevil in both growing seasons, but it was susceptible to stink bug feeding. Abel et al. (2000) assessed 15 experimental maize lines for leaf, ear, and stalk feeding of four lepidopteran insects, the fall armyworm, the corn earworm, the southwestern corn borer, *Diatraea grandiosella* Dyar, and the sugarcane borer, *Diatraea saccharalis* (F.). They reported that maize resistance to insects differed among the lines tested; however, no lines conferred resistance to multiple insects. It is intriguing to observe resistance to both chewing and sucking insects by these inbred lines as well as the two experimental hybrids in current study. This finding merits further examination of the parental inbred lines to understand biochemical and/or physiological mechanisms that might have contributed to plant resistance to both chewing and piercing–sucking ear-feeding insects. The identification of a corn inbred line that confers resistance to multiple insect pests would be valuable for corn breeding programs.

The corn silk-feeding bioassay showed that the corn silk from the population with high maysin levels reduced the growth of both fall armyworm and corn earworm. This supports the findings by Waiss et al. (1979), Wiseman et al. (1983), Wiseman and Isenhour (1990), and Wiseman et al. (1992) that the allelochemical in silk, maysin, contributed to the antibiotic resistance to silk-feeding insects. Wiseman et al. (1983) found that 0.2% of maysin of fresh weight could result in a 50% weight reduction in corn earworm, and >60% of weight loss in the fall armyworm. Maysin is a flavone glycoside that exists in grasses such as corn, teosinte, and centipede grass (Gueldner et al. 1991). The corn earworm is a polyphagous herbivore, and it has a versatile detoxification system, which could catabolize allelochemicals such as maysin more efficiently than that in the fall armyworm. Furthermore, our silk bioassay also demonstrated that the corn silk from SIM6/EPM6 combination conferred antibiotic resistance to both fall armyworm and corn earworm by significantly reducing the larval weight of both species of the insects. In contrast, the corn silk from Hytest7930Bt did not affect fall armyworm growth, but virtually stopped the growth of the corn earworm larvae. This intriguing difference could be the result of the specific event of the *B. thuringiensis* gene inserted in Hytest 7930Bt only targeting the corn earworm, but had minimal effect on the fall armyworm. Our current observations were similar to the report by Buntin et al.

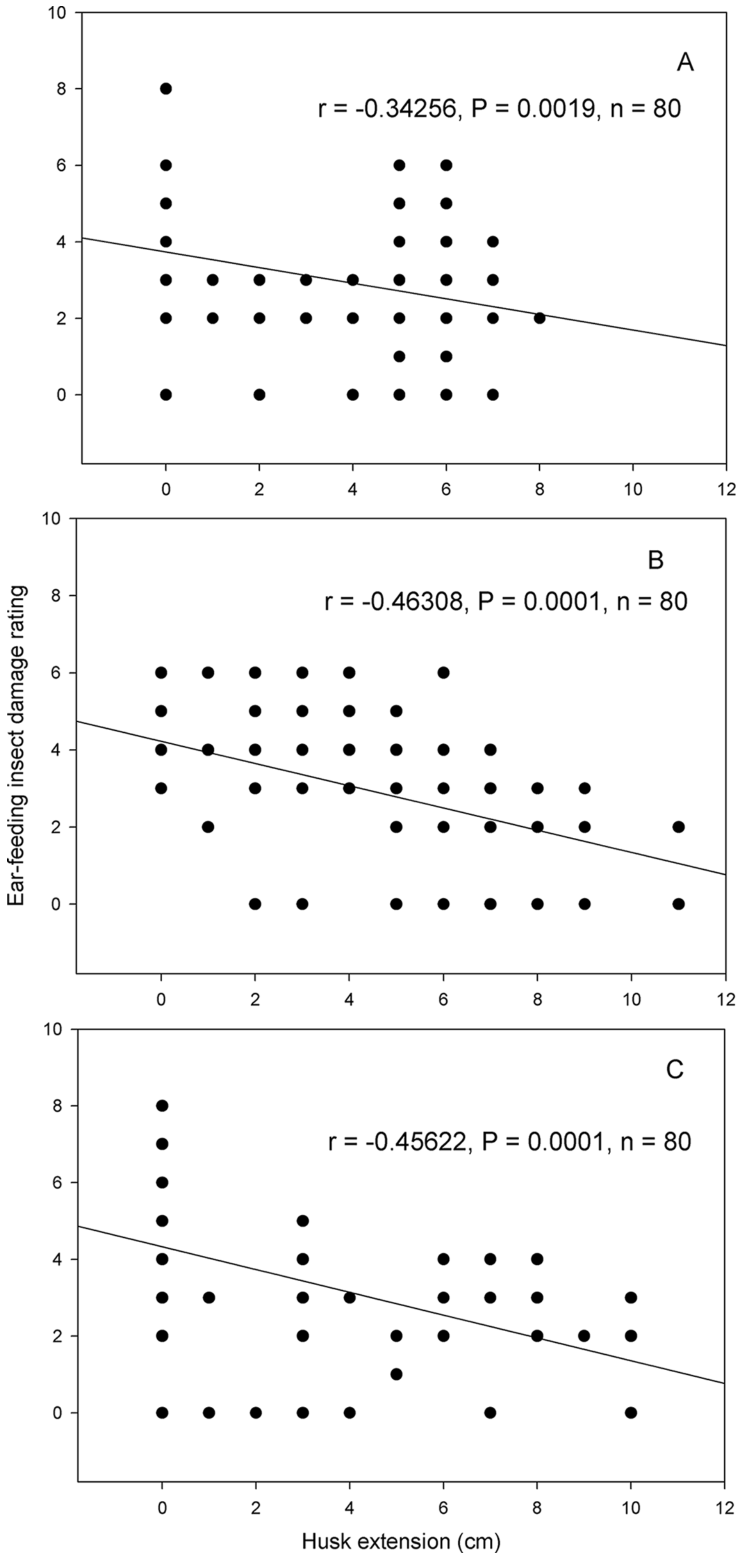

Fig. 4. Negative correlation between husk extension (centimeters) and corn earworm damage ratings on the three corn hybrids. (A) Corn hybrid C3A654-1*B110. (B) Corn hybrid C3S1B73-3*Tx205. (C) Corn hybrid SGP3*C3S1B73-3. The graphs showed less data points than the sample sizes ($n = 80$), which was caused by the overlapping data points in the data sets.

(2004). They reported that the effectiveness of Bt-transgenic corn hybrids in general were usually tissue (or site)-specific expression of the toxin. Another possibility would be that differential susceptibility of corn earworm and fall armyworm to a specific event of Bt gene expressed in the hybrid Hytest 7930Bt might be the cause of the difference in larval mortality shown in the silk-feeding study. The finding merits further examination in future studies.

Previous studies on husk tightness in corn described a negative correlation to corn earworm damage (Barry et al. 1986, Wiseman and Isenhour 1992, Rector et al. 2002). We observed that corn husk extension also was negatively correlated to corn earworm damage on three of the 10 hybrids, but not on any of the 10 inbred lines. Similarly, Bernabe-Adalla and Bernado (1976) found that maize weevil damage is negatively correlated to corn husk extension, which is similar to our findings using the experimental hybrids. Moreover, we demonstrated that corn husk extension could mitigate the damage by both corn earworm and maize weevil in the field, although significant differences between years indicates that this trait may be influenced by environmental conditions. In addition, reduced damage on C3A654-1*B110 and C3S1B73-3*Tx205 with short husk extension suggests that these hybrids might contain another mechanism for insect resistance such as high levels of allelochemical(s) in silks, cobs, or kernels that confer antibiotic resistance.

The inheritance and contribution of various chemical (i.e., corn silk maysin and kernel phenolics), and physical (e.g., corn husk coverage) traits of corn ears have been examined for either corn earworm (Widstrom and Snook 1998, 2001a. 2001b, 2001c; Guo et al. 1999; Rector et al. 2002) or maize weevil (Widstrom et al. 1974, Arnason et al. 1992, Kim and Kossou 2003), respectively. However, the genetics of corn resistance to multiple insects still merits further examination. In addition, the information about the influence of agronomic practices (i.e., planting dates) and environmental factors on insect damage also have been assessed for corn earworm (Wiseman and Isenhour 1992) and maize weevil (Brown and Lee 2002). Information about genetics and agronomic practices of corn–insect pest interactions would allow us to further develop and deploy new corn germplasm that confers multiple ear-feeding insect resistance. This study suggested that the development of new corn hybrids with innate multiple-insect resistance had the potential to strengthen and complement transgenic Bt technology. The pyramiding of innate corn traits that confer multiple insect resistance in addition to Bt and other transgenic technologies could offer a synergistic impact on corn production in southern United States. Such advancement in management of ear-feeding insect damage and mycotoxin (e.g., aflatoxin and fumonisin) contaminations in corn production is critical to meet the overwhelming demand of quality corn for animal feed and biofuels.

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